

9.—THE ASCORBIC ACID CONTENT OF SOME WESTERN AUSTRALIAN FRUITS

(AS INDICATED BY TITRATION WITH PHENOL-INDO-2:6-DICHLOROPHENOL).

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This paper details the results of determinations of ascorbic acid (vitamin C) in a number of Western Australian fruits during the season 1937-38. The object of the investigation was to make a rapid survey of the principal fruits which are grown in the State, firstly to discover whether the values found are comparable with those reported elsewhere for the same fruits, and, secondly, to record values for certain fruits which do not appear to have been examined elsewhere. In all cases, except where otherwise stated, fruits freshly gathered on the day on which the analyses were made have been utilised. Choice, ripe specimens only were used, and the analyses were completed as quickly as possible.

It is recognised that each individual kind of fruit has a range of values of ascorbic acid content over which variation occurs. For this reason it is not claimed for these results, obtained as they are from two or three and in some cases one sample only, that they should serve as any more than an indication for reference, or for future use in determining the ranges and means.

Method.—During recent years a number of workers have made determinations of the ascorbic acid content of various biological materials, particularly vegetable products such as fruits, as well as extracts of animal tissues. This work has been stimulated by findings regarding the importance of vitamin C in certain conditions and disorders of the human subject in addition to the rôle which it plays as the antiscorbutic factor in nutrition. It is recognised that the most accurate method of determining the ascorbic acid is the biological assay method of feeding to animals such as guinea pigs, in which the effects of the substance under examination are compared with those of a prepared food containing a known amount of the vitamin. This method is long and tedious and, moreover, cannot be carried out without the resources of an animal laboratory, which are not available in Western Australia.

The determination of ascorbic acid has been considerably facilitated, however, by the introduction by Tillmans and his associates (1) and development by Birch, Harris & Ray (3) of a simple titration procedure taking advantage of the reducing power of the acid and using the oxidation-reduction indicator phenol-indo-2:6-dichlorophenol. The method is supported by a considerable amount of animal assay work and, with minor modifications, has come into extensive use. Bessey & King (4) and others have pointed out that ascorbic acid is not an absolutely specific reducing agent for the indicator, as other substances exist in biological materials, such as glutathione, cysteine, phenolic compounds, etc., which have a lower reduction potential and are, therefore, a possible source of interference. The magnitude of the resultant error is greatest in materials with a low ascorbic acid content, such as animal tissues and extracts which, moreover, contain more

of the interfering substances. With vegetable tissues, particularly fruit juices and extracts, the percentage error is much less, being usually of the order of 2 to 3 per cent. The method, therefore, is well suited for a survey of this nature, involving only fruits, where an indication of the degree of the ascorbic acid content rather than the exact amount is required.

The method used in this work was essentially that described by Bessey & King (5). From one to six fruits, depending on the size, were used. The edible portion only was taken. A suitable quantity, usually 10 or 20 grams, was rapidly ground in a mortar with sand and 8 per cent. trichloroacetic acid, centrifuged, and the extract poured off, then ground with more acid and centrifuged again. The combined extracts were made to 50 ml and an aliquot of 10 or 20 ml taken for titration. A 0.05 per cent. solution of phenol-indo-2:6-dichlorophenol was run in until a definite pink colour was obtained. This preliminary titration gave the approximate amount of the dye required. A second titration was made by running in most of the quantity required and adding the remainder slowly, the whole titration usually being complete in two minutes. The conditions stressed by Harris (6), namely, removal of proteins by trichloroacetic acid, extraction with as little delay as possible and titration at an acid reaction in a time not exceeding two minutes, were thus all complied with.

The phenol-indo-2:6-dichlorophenol solution was standardised periodically against pure ascorbic acid (B.D.H.). The method of standardisation against the iodine titration of fresh lemon juice, advocated by Bessey & King, was also found satisfactory.

In the case of fruits yielding coloured extracts to trichloroacetic acid, such as mulberry, plum and fig, the modification by McHenry & Murray Graham (7) of the method described by Tillmans, Hirsch & Jackisch (2), involving titration in the presence of a layer of chloroform, which dissolves the red colour of the excess of dye but not the anthocyanins of the fruits, was used with success.

The results, giving in the following table, are arranged in approximately descending order of the ascorbic acid content, citrus fruits and tropical fruits, however, being separated from the others.

	Locality.	Ascorbic acid. Mg. per gm.
<i>Citrus Fruits.</i>		
Orange (<i>Citrus sinensis</i> Osbeck)—		
Washington navel	Gosnells	0.49
		0.39
Valencia	Gosnells	0.68
		0.44
Mandarin orange (<i>Citrus nobilis</i> Lour var. <i>deliciosa</i> Swingle)	Gosnells	0.51
Lemon (<i>Citrus limonia</i> Osbeck)	Claremont	0.43
	Gosnells	0.33
	s.p.*	0.42
Kumquat (<i>Fortunella japonica</i> Swing)	South Perth	0.30
Grape fruit or pummelo (<i>Citrus maxima</i> Merr.)	Gosnells	0.33
Lime—New Caledonian (<i>Citrus aurantifolia</i> Swingle)	South Perth	0.26
Citron—Sport from orange (<i>Citrus medica</i> L.)	Claremont	0.24

*s.p.—Purchased from shops.

Note.—The botanical names are taken from L. H. Bailey's "Manual of Cultivated Plants."

	Locality.	Ascorbic acid. Mg. per gm.
<i>Miscellaneous Fruits.</i>		
Banana passion fruit (<i>Passiflora mollissima</i> Bailey)	s.p.*	0.44
Passion fruit (<i>Passiflora edulis</i> Sims)	s.p.	0.24
	Osborne Park	0.24
Cape Gooseberry (<i>Physalis peruviana</i> L.)	s.p.	0.22
		0.21
Peach (<i>Prunus Persica</i> Sieb u Zucc.)—		
Early variety	s.p.	0.01
Elberta	Gosnells	0.17
Apricot (<i>Prunus Armeniaca</i> L.)	s.p.	0.06
Mulberry (<i>Morus nigra</i> L.)	Claremont	0.13
	Cottesloe	0.08
Rock-melon (<i>Cucumis Melo</i> L.)	Gosnells	0.10
Water-melon (<i>Citrullus vulgaris</i> Schrad)	Upper Swan	0.14
	Upper Swan	0.05
Plum (<i>Prunus domestica</i> L.)—		
Satsuma	Gosnells	0.07
	Karragullen	0.03
Nectarine (<i>Prunus Persica</i> Sieb u Zucc. var. <i>nucipersica</i> Schneid)	Karragullen	0.08
Grape (<i>Vitis vinifera</i> L.)—		
Xante currant	Claremont	0.03
Muscat-Gordo Blanco	Claremont	0.04
Muscat of Alexandria	Claremont	0.05
Muscat Canon Hall (partly ripe)	South Perth	0.04
Sultana	Claremont	0.06
Black Prince	Claremont	0.05
Crystal	Cottesloe	0.02
Fig (<i>Ficus carica</i> L.)—		
Adam (cross)	South Perth	0.02
Adam	Cottesloe	0.02
Brown Turkey	Cottesloe	0.02
Smyrna	Applecross	0.03
Loquat (<i>Eriobotrya japonica</i> Lindl.)	Gosnells	<i>nil</i>
	s.p.	<i>nil</i>
<i>Tropical Fruits.</i>		
Guava—Large yellow (<i>Psidium Guajava</i> L.)	s.p.	1.10
Papaw (<i>Carica papaya</i> L.)	Carnarvon	0.98
Rock-melon—Honeydew variety (<i>Cucumis Melo</i> L. var. <i>inodorus</i> Waud.)	Carnarvon	0.21
Pineapple (<i>Ananas comosus</i> Merr.)	Carnarvon	0.19
Mango (<i>Mangifera indica</i> L.)	Carnarvon	0.13
Banana (<i>Musa paradisiaca</i> L. var. <i>sapientum</i> Kuntze)—		
Cavendish	Carnarvon	0.12
Sugar	Carnarvon	0.64
Plantain	Carnarvon	0.11
Golden Gros	Carnarvon	0.02

*s.p.—Purchased from shops.

Note.—The botanical names are taken from L. H. Bailey's "Manual of Cultivated Plants."

The values found for those fruits which have been examined elsewhere are seen to be well in accord with other reported results. The high values for guava and papaw are noteworthy, whilst those for the banana passion fruit, passion fruit and cape gooseberry, which do not appear in the literature as having previously been examined, indicate that these fruits are moderately good sources of ascorbic acid. Another fruit not noted pre-

viously is the mulberry which, although a very juicy fruit, is not a good source. Grapes and figs have a very low value. The complete absence of even a trace in the loquat is also an interesting result.

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REFERENCES.

- (1) 1932—Tillmans, J., Hirsch, P. & Hirsch, W.: *Z. Untersuch Lebens*: 1932.63.1. Abstract Analyst 1932.57.260.
- (2) 1932—Tillmans, Hirsch & Jackisch: *Z. Untersuch Lebens*: 1932.63.241. Abstract Analyst 1932.57.396.
- (3) Birch, Harris & Ray: *Biochem. J.*, 1933. 27, I., 590.
- (4) Bessey and King: *J. Biol. Chem.*, 1933, 103, 687.
- (5) Bessey and King. *Id.*, 1933, 103, 690.
- (6) Harris: *5th Internat. Tech. & Chem. Congress Agricult. Industries*.
- (7) McHenry & Murray Graham: *Biochem. J.*, 1935, 29, II., 2013.

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